SUMMARY

The development of different ideas concerning the multiplicity of haemoglobin among species and individuals has been outlined in the introductory part.

Chapter I covers a short review of the literature with regard to the numerous investigations which have lead to the current concepts of the structure of the haemoglobin molecule in general and concerning the search for structural differences between specific haemoglobins.

The methods used in this investigation are described in detail in chapter II, in which a scheme of the general procedure that was followed in order to obtain comparable results for the several haemoglobin structures is also given.

Chapter III deals with the investigations of normally occurring adult and foetal human haemoglobins. Comparison of these haemoglobins was performed at three stages. The native haemoglobins were examined by chromatography, mainly on carboxymethylcellulose which also enabled the isolation of purified components. The polypeptide chains of haemoglobins were compared by electrophoresis and chromatography of the corresponding globins at low pH, the latter method being used for their isolation. At the third level, a comparison of the fingerprints of the tryptic peptides of both corresponding haemoglobins and their subunits was made.

Haemoglobins of several animals, including cattle, sheep, goat, rabbit, hare and chicken were examined at the same molecular and submolecular levels as the human haemoglobins. The results are described in chapter IV.

In the last chapter, the results obtained from human and animal haemoglobins are discussed in the light of modern concepts of genetics and certain conclusions have been put forward.

To summarize the results, it was observed in agreement with other investigators that in most species haemoglobin is heterogeneous.

Evidence was presented that the heterogeneity in all the species investigated is partly due to secondary factors which means that the haemoglobin molecule is altered after its completion. It is highly likely that the alteration is caused by the binding of one or more molecules of glutathione to a haemoglobin molecule in a manner which is not quite understood yet.

Of the normal human haemoglobins, three components (A_1, A_2 and F_1) appear to be distinct biological entities, that is, they are synthesized by at least three different synthesizing systems. The three molecules are composed of four different types of polypeptide chains, α-, β-, γ- and δ-chains. Hb-A_1 consists of α- and β-chains, Hb-F_1 of α- and γ-chains and Hb-A_2 of α- and δ-chains. Like the human haemoglobins, the haemoglobins of cattle, sheep, goat and rabbit were also found to contain a common chain type in their adult and foetal components. This suggests that the principle of using partly the same subunits in building different molecules is a general one.

Two genetically controlled different adult ovine haemoglobins have one chain type in common which is also shared by the foetal component. It is highly likely that the differences between the other two chain types are polytopical. This situation is quite different from what is observed in the case of normal and abnormal human haemoglobins, where only single amino acid substitutions have been detected with certainty so far.

The two haemoglobins normally present in chicken blood were found to be composed of four different chain types. These blood pigments do not seem to possess common chains.

The fingerprints of the mammalian and avian haemoglobins and their subunits enabled the comparison of the haemoglobin structures of different species. Inspection of the peptide patterns revealed that in spite of several differences, the subunits of each of the mammalian haemoglobins were homologous either to the α-chains or to the β-chains of human haemoglobin. Moreover, evidence was presented that the differences between two homologous peptide chains exist in a number of single amino acid substitutions scattered over the full length of the chain.

The peptide patterns suggest that less structural differences exist between haemoglobins of species with a close common ancestry than between haemoglobins of animals that evolve independently during longer times. A clear-cut homology of mammalian and avian haemoglobins could not be demonstrated by fingerprinting. It is

assumed that the comparability is diminished as a consequence of the remote phylogenetic relationship of these animal classes.

Attention was drawn to the presence of a certain region in the polypeptide chains of all the haemoglobins investigated. This part appears to be extremely resistant against mutational alterations and might be of importance for the function of the molecule.